BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Research

Cite this article: Staats EG, Agosta SJ, Vonesh JR. 2016 Predator diversity reduces habitat colonization by mosquitoes and midges. *Biol. Lett.* **12**: 20160580.
http://dx.doi.org/10.1098/rsbl.2016.0580

Received: 11 July 2016
Accepted: 2 December 2016

Subject Areas:
ecology, behaviour

Keywords:
multiple predator effects, non-consumptive effects, oviposition habitat selection

Author for correspondence:
Ethan G. Staats
e-mail: egstaats6@msn.com

THE ROYAL SOCIETY PUBLISHING

Community ecology

Predator diversity reduces habitat colonization by mosquitoes and midges

Ethan G. Staats[1], Salvatore J. Agosta[2] and James R. Vonesh[1,3]

[1]Department of Biology, and [2]Center for Environmental Sciences, Virginia Commonwealth University, Richmond, VA, USA
[3]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

EGS, 0000-0002-5801-6756

Changes in predator diversity via extinction and invasion are increasingly widespread and can have important ecological and socio-economic consequences. Anticipating and managing these consequences requires understanding how predators shape ecological communities. Previous predator biodiversity research has focused on post-colonization processes. However, predators can also shape communities by altering patterns of prey habitat selection during colonization. The sensitivity of this non-consumptive top down mechanism to changes in predator diversity is largely unexamined. To address this gap, we examined patterns of dipteran oviposition habitat selection in experimental aquatic habitats in response to varied predator species richness while holding predator abundance constant. Caged predators were used in order to disentangle behavioural oviposition responses to predator cues from potential post-oviposition consumption of eggs and larvae. We hypothesized that because increases in predator richness often result in greater prey mortality than would be predicted from independent effects of predators, prey should avoid predator-rich habitats during colonization. Consistent with this hypothesis, predator-rich habitats received 48% fewer dipteran eggs than predicted, including 60% fewer mosquito eggs and 38% fewer midge eggs. Our findings highlight the potentially important links between predator biodiversity, prey habitat selection and the ecosystem service of pest regulation.

1. Introduction

Species losses and introductions dramatically alter the diversity of ecological communities, including the abundance and distributions of predators and prey. Understanding the consequences of these changes requires a better understanding of the ways predators affect community structure, and ecosystem function and services such as the regulation of pest species. Past research on predator diversity and prey suppression has focused primarily on closed experimental systems where the effects of predators are measured after prey have colonized or been experimentally added to a habitat. This work highlights that the combined effects of multiple predator species are often difficult to predict from the independent effects of the constituent species [1] and that increased predator diversity often results in reduced prey abundance (reviewed by Griffin *et al*. [2]).

By focusing on post-colonization processes, past research on the effects of predator biodiversity has implicitly assumed that patterns of prey abundance result from consumption by predators, and thus that prey colonize habitat, and community structure builds randomly with respect to predators. However, there is considerable evidence to the contrary; many organisms actively assess risk during habitat selection [3–7]. The failure to address non-consumptive predator

biodiversity effects on habitat selection is an important gap in our understanding of predator effects, especially considering habitat selection necessarily occurs prior to post-colonization processes and therefore predator effects on habitat selection may alter subsequent effects [8,9].

How should prey select habitat in response to predator diversity? Adaptive response to predators requires that prey can accurately assess risk. In natural multiple predator communities, adaptive habitat selection may represent an evolutionary challenge as consumption by combined predator species is often difficult to predict from consumption by constituent species independently [1,2]. While predicting the effect of combined predators is difficult, past research suggests that across systems and taxa increased predator diversity often results in increased prey suppression [2]. Therefore, we hypothesized that habitat selection would also be sensitive to changes in predator diversity, and we predicted stronger avoidance of more diverse predator assemblages. We tested this prediction by examining the oviposition habitat selection (OHS) response of dipteran insects colonizing experimental habitats of varied predator richness, but constant predator abundance.

2. Material and methods

This study was conducted July–August 2014 at the Virginia Commonwealth University Rice Rivers Center (37.19′55 N, 77.12′21 W; http://www.vcu.edu/rice/). We quantified mosquito and midge OHS in response to invertebrate predators: larval Halloween pennant dragonflies (*Celithemis eponina*), larval bluet damselflies (*Enallagma* spp.) and first-year *Procambarus* crayfish (*Procambarus* spp.). These taxa are seasonally abundant in James River rock pools near Richmond, Virginia and use different foraging modes and microhabitats [10,11], increasing the likelihood that their combined effects will result in greater prey suppression [12]. We conducted this experiment in 25 aquatic mesocosms (38 l Sterilite® containers) filled with river water and 3 l leaf litter (litter composition detailed by Bellile & Vonesh [13]). Mesocosms were arrayed in a square grid with 3 m between containers along the edge of old field and forest habitats.

We randomly established five predator treatments: no predator, three separate low richness treatments each with three individuals of one predator type and high richness with one individual of each type. Each treatment was replicated five times each in two time blocks, for a total of 50 experimental units. We caged predators individually in screened enclosures to prevent egg clutch consumption and intra-guild predation. Caged predators were fed equal numbers [14] of *Culex* spp. mosquito larvae every other day to generate predator cues (3–12 per feeding). We counted and removed egg clutches of colonizing dipterans floating on the water surface every other day for 8 days.

To test for the effect of predator richness on dipteran colonization, we used a pair of complementary approaches. First, we tested whether the observed oviposition in the predator-rich treatment differed from predicted oviposition. Predicted oviposition was calculated as the average of the low richness treatments, a common approach [1] that assumes a linear relationship between predator richness and prey oviposition. Second, treating predator richness as a continuous predictor, we tested whether observed oviposition declined with increasing predator richness. We also tested for differences in prey responses to specific individual predator species by comparing oviposition across all five predator treatments. We accomplished these analyses using Poisson log-link generalized linear model (GLM) followed by Tukey's post hoc tests (where appropriate). Temporal block and the distance of the mesocosm from the forest edge were included as covariates in our analyses.

3. Results

A total of 188 mosquito egg masses, including 151 *Culex* spp. and 37 *Anopheles* spp. masses, were oviposited in the experiment. Previous mesocosm field studies show that colonizing *Culex* at this location were 99% *C. restuans* and 1% a combination of *C. quinquefasciatus* complex and *C. territans* [15]. A total of 193 chironomid midge egg masses were oviposited. Past studies at this site show that more than 90% colonizing chironomids were *Chironomus* spp. [9]. In addition, 11 dipteran egg masses that were oviposited could not be identified further.

Predators had strong effects on colonization. *Culex* spp. (figure 1*a*), chironomid (figure 1*c*) and dipteran oviposition (figure 1*e*) differed across treatments (table 1). Furthermore, *Culex* (figure 1*b*), chironomid (figure 1*d*) and dipteran oviposition (figure 1*f*) all decreased with increasing predator richness (table 1). *Culex* oviposition in the high richness treatment was 60% lower than predicted (figure 1*a*; $Z = -2.224$, d.f. $= 39$, $p = 0.0262$). *Culex* oviposition declined 51% in response to dragonflies and 45% in response to crayfish, relative to the predator-free control, and declined 69% in the high richness treatment relative to the damselfly treatment (table 1 and figure 1*a*). *Culex* oviposition declined 76% across the range of predator richness (figure 1*b*). Chironomid oviposition in the high richness treatment was 38% lower than predicted (figure 1*c*; $Z = -2.127$, d.f. $= 39$, $p = 0.0334$). Chironomid oviposition declined 43% in response to damselflies, relative to the predator-free control, and declined 50% in the high richness treatment relative to the crayfish treatment (table 1 and figure 1*c*). Chironomid oviposition declined 55% across the range of predator richness (figure 1*d*). Total dipteran oviposition in the high richness treatment was 48% lower than predicted (figure 1*e*; $Z = -3.851$, d.f. $= 39$, $p = 0.0001$). Total dipteran oviposition declined 37% in response to dragonflies, 31% to damselflies and 27% to crayfish, relative to predator-free control, and declined 44%, 48% and 51% in the predator-free control relative to dragonfly, damselfly and crayfish treatments, respectively (table 1 and figure 1*e*). Total dipteran oviposition declined 64% across the range of predator richness (figure 1*f*).

4. Discussion

Here, we show that mosquitoes and midges reduce oviposition in response to specific predators and that this alters the assembly of aquatic dipteran communities. Further, holding predator abundance constant, we see prey oviposition decreases with increasing predator species richness and that the community-level response to rich predator assemblages was stronger than would be predicted from the responses to individual predators. Increasing avoidance with increasing predator diversity could arise through sampling effects, where the likelihood of including dominant taxa increases as a direct result of increased richness, or through synergism where the response to combined predators is greater than predicted from responses to individual predators and is greater than the response to any single predator species. The total dipteran response suggests combined predators may affect prey oviposition in a synergistic manner. However, mosquitoes and midges separately showed responses to high richness mesocosms not significantly different from some single predator treatments, so the possibility of sampling effects cannot be ruled out. Importantly, these

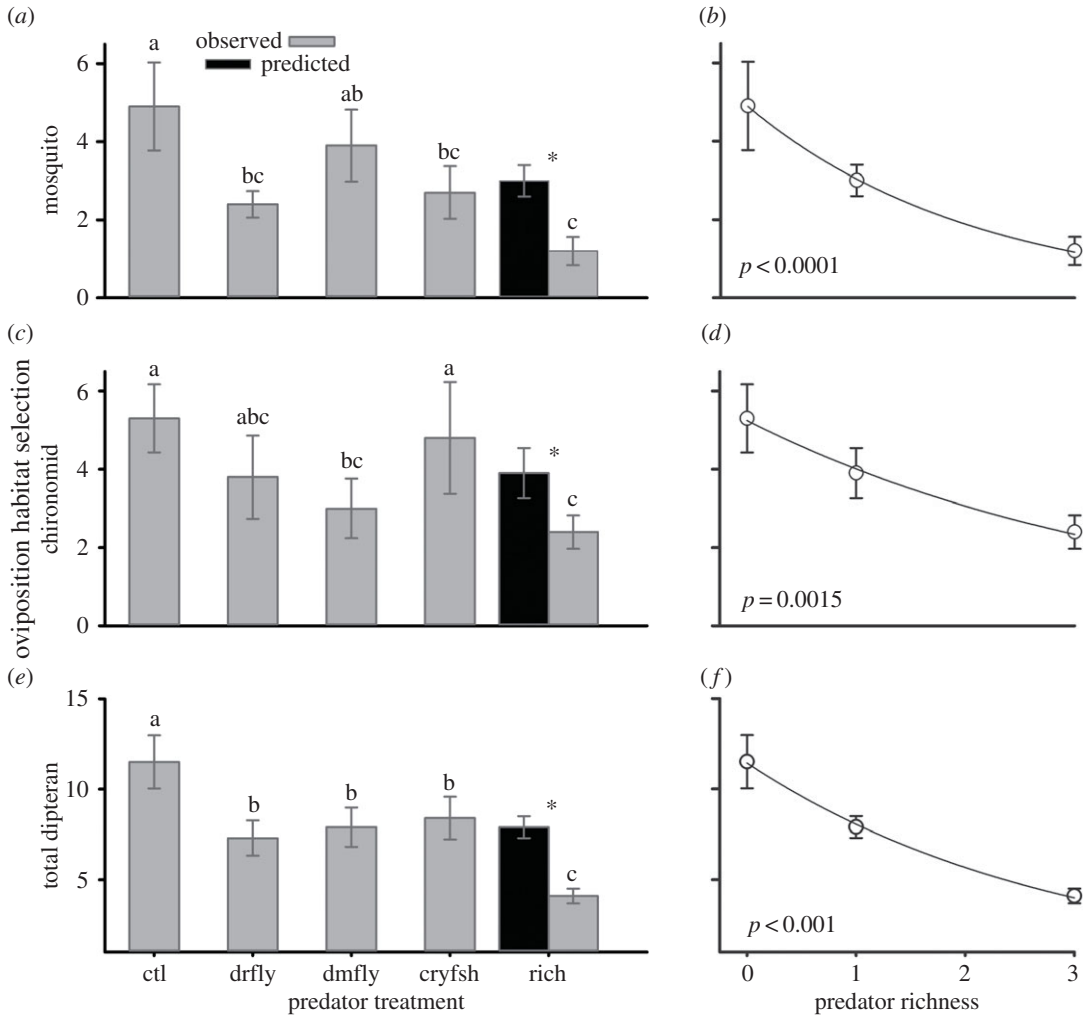

Figure 1. Bar graphs show means and standard errors for number of mosquito (*a*), midge (*c*) and total dipteran (*e*) egg clutches oviposited per mesocosm per time block among all treatments (ctrl, predator-free control; drfly, dragonfly; dmfly, damselfly; cryfsh, crayfish; and rich, high richness) and the predicted high richness response. Letters above bars mark significant differences among all five predator treatments, while asterisks mark where the observed and predicted high richness responses differ. Point-and-whisker plots show mean and standard error for number of egg clutches of mosquitoes (*b*), midges (*d*) and total dipterans (*f*) per mesocosm per time block along a gradient of predator species richness (0, predator-free control ($n = 10$); 1, averaged single-species treatments ($n = 30$); 3, high richness ($n = 10$)).

Table 1. Treatment and predator richness effects given from Poisson log-link GLMs on oviposition by mosquitoes, midges and dipterans. Treatment effect GLMs test the effects of categorical treatment on prey oviposition among predator-free controls, low richness and high richness treatments. Richness effect GLMs tested whether there is a significant negative relationship between continuous predator richness and oviposition. GLMs for *Culex* oviposition contained distance from the forest edge and time block as covariates, and for chironomids contained time block as a covariate.

	treatment effects			richness effects			
	F	d.f.	*p*-value	*Z*	d.f.	*p*-value	slope (s.e.)
Culex	28.15	4	<0.0001	−4.18	49	<0.0001	−0.45 (0.109)
Chironomidae	15.41	4	0.0039	−3.18	49	0.0015	−0.27 (0.083)
Diptera	37.33	4	<0.0001	−5.69	49	<0.0001	−0.35 (0.061)

predator-mediated effects on oviposition occur prior to any consumptive effects on prey and will act as an initial filter to community assembly.

Why did prey more strongly avoid our high richness treatment? We can think of three possible mechanisms. First, recent meta-analyses show that on average more diverse predator assemblages result in increased prey suppression, i.e. greater risk to prey [2]. Generally avoiding predator-rich habitats may thus be an adaptive oviposition strategy, even if prey are unable to accurately assess the specific risk of a particular predator assemblage. Alternatively, prey may be able to respond adaptively to risk presented by specific predator assemblages. Avoidance, then, should increase only for specific predator assemblages that exhibit synergistic combined predation, and prey should be neutrally affected by, or attracted to antagonistic predator assemblages. This would require prey to be able to finely assess risk. Some prey do show specific responses to predators [16,17] and can integrate multiple cues during colonization [5]. Therefore, fine-tuned assessment of risk in response to specific predator assemblages is possible.

Lastly, stronger avoidance with increasing richness could arise if (i) oviposition declines nonlinearly (e.g. negative exponentially) with increasing abundance of individual predator taxa [18,19] and (ii) responses to different predator taxa combine independently. If both conditions hold, the addition of individuals of unique predator taxa would likely yield stronger avoidance by prey than subsequent additions of individuals of the same predator taxon. Distinguishing among these mechanisms will require experiments with multiple predator-rich assemblages and an understanding of the shape of the predator abundance/oviposition avoidance relationship.

Non-consumptive interactions between predators and their prey are appreciated as a key factor shaping aquatic communities and their functions [20,21]. This experiment extends previous research demonstrating that non-consumptive effects of predator species richness may alter colonization patterns, influencing community assembly and structure [8,9]. Interestingly, previous work in some instances may have confounded predator consumption with predator avoidance, as both produce the same pattern, lower prey abundance. However, there are important distinctions. Increasing prey avoidance with increasing diversity may locally reduce prey abundance, but colonization may be redirected elsewhere regionally [22]. While previous research may have highlighted that increasing predator diversity can increase prey mortality [2], here we show that in an open system increased diversity resulted in increased avoidance of predator patches and thus may actually reduce predation. While further research examining the mechanisms underlying our observations is needed, our results highlight the potential importance of predator diversity in shaping community assembly, and ecosystem services such as pest regulation by altering patterns of prey habitat selection.

Data accessibility. All data used in all analyses are available on the Dryad data repository: http://dx.doi.org/10.5061/dryad.2f452 [23].

Authors' contributions. S.J.A. and J.R.V. advised E.G.S. as mentors during this research. E.G.S., S.J.A. and J.R.V. agree to be held accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. E.G.S., S.J.A. and J.R.V. testify that this manuscript has neither been published, in whole or in part, nor is it under consideration for publication elsewhere, and that all authors have been actively involved in work and the manuscript. E.G.S. and J.R.V. conceived the idea. E.G.S., S.J.A. and J.R.V. developed the methods. E.G.S. conducted fieldwork. E.G.S., S.J.A. and J.R.V. performed statistical analyses. E.G.S., S.J.A. and J.R.V. wrote the manuscript. E.G.S., S.J.A. and J.R.V. contributed to drafting and revising the article, and all approve the final submitted version.

Competing interests. We declare we have no competing interests.

Funding. This study was partially supported by a Rice Rivers Center student research grant to E.G.S. The US Fulbright Program and the DST-NRF Centre of Excellence for Invasion Biology at Stellenbosch University provided support to J.R.V. during writing.

Acknowledgements. We thank J. Neale, T. Price, K. Tran, N. Cason and R. Lidder for help with fieldwork; and L. Bulluck, A. Garey and D. McGarvey for advice with experimental design and writing. This is VCU Rice Rivers Center publication no. 76.

References

1. Sih A, Englund G, Wooster D. 1998 Emergent impacts of multiple predators on prey. *Trends Ecol. Evol*. **13**, 350–355. (doi:10.1016/S0169-5347(98)01437-2)
2. Griffin JN, Byrnes JE, Cardinale BJ. 2013 Effects of predator richness on prey suppression: a meta-analysis. *Ecology* **94**, 2180–2187. (doi:10.1890/13-0179.1)
3. Resetarits Jr WJ, Wilbur HM. 1989 Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. *Ecology* **70**, 220–228. (doi:10.2307/1938428)
4. Vonesh JR, Blaustein L. 2010 Predator-induced shifts in mosquito oviposition site selection: a meta-analysis and implications for vector control. *Isr. J. Ecol. Evol*. **56**, 263–279. (doi:10.1560/IJEE.56.3-4.263)
5. Walzer A, Schausberger P. 2012 Integration of multiple intraguild predator cues for oviposition decisions by a predatory mite. *Anim. Behav*. **84**, 1411–1417. (doi:10.1016/j.anbehav.2012.09.006)
6. Wesner JS, Meyers P, Billman EJ, Belk MC. 2012 Multiple predators indirectly alter community assembly across ecosystem boundaries. *Ecology* **93**, 1674–1682. (doi:10.1890/11-2061.1)
7. Wesner JS, Meyers P, Billman EJ, Belk MC. 2015 Habitat selection and consumption across a landscape of multiple predators. *Ecol. Evol*. **5**, 121–129. (doi:10.1002/ece3.1256)
8. Vonesh JR, Kraus JM, Rosenberg JS, Chase JM. 2009 Predator effects on aquatic community assembly: disentangling the roles of habitat selection and post-colonization processes. *Oikos* **118**, 1219–1229. (doi:10.1111/j.1600-0706.2009.17369.x)
9. Kraus J, Vonesh JR. 2010 Feedbacks between assembly and top predator-mediated habitat selection shape variation in local colonization. *J. Anim. Ecol*. **79**, 795–802. (doi:10.1111/j.1365-2656.2010.01684.x)
10. Holdich DM *et al.* 2002 *Biology of freshwater crayfish*. Osney Mead, Oxford, UK: Blackwell Science Ltd.
11. Merritt RW, Cummins KW, Berg MB. 2008 *An introduction to the aquatic insects of North America*, 4th edn. Dubuque, IA: Kendall/Hunt Publishing Company.
12. Finke DL, Snyder WE. 2008 Niche partitioning increases resource exploitation by diverse communities. *Science* **321**, 1488–1489. (doi:10.1126/science.1160854)
13. Bellile K, Vonesh J. 2016 Bioinsecticide and leaf litter combination increases oviposition and reduces adult recruitment to mesocosm to create an effective ovitrap for *Culex* mosquitoes. *J. Vector Ecol*. **138**, 300–305.
14. McCoy MW, Touchon JC, Landberg T, Warkentin KM, Vonesh JR. 2012 Prey response to predator chemical cues: disentangling the importance of the number and biomass of prey consumed. *PLoS ONE* **7**, e47495. (doi:10.1371/journal.pone.0047495)
15. Kraus JM, Vonesh JR. 2012 Fluxes of terrestrial and aquatic carbon by emergent mosquitoes: a test of controls and implications for cross-ecosystem linkages. *Oecologia* **170**, 1111–1122. (doi:10.1007/s00442-012-2369-x)
16. Henry LM, Bannerman JA, Gillespie DR, Roitberg BD. 2010 Predator identity and the nature and strength of food-web interactions. *J. Anim. Ecol*. **79**, 1164–1171. (doi:10.1111/j.1365-2656.2010.01723.x)
17. Miller JRB, Ament JM, Schmitz OJ. 2014 Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *J. Anim. Ecol*. **83**, 214–222. (doi:10.1111/1365-2656.12111)
18. Rieger JF, Binckley CA, Resetarits Jr WJ. 2004 Larval performance and oviposition site preference along a predation gradient. *Ecology* **85**, 2094–2099. (doi:10.1890/04-0156)
19. Eitam A, Blaustein L. 2004 Oviposition habitat selection by mosquitoes in response to predator (*Notonecta maculata*) density. *Physiol. Entomol*. **29**, 188–191. (doi:10.1111/j.0307-6962.2004.0372.x)
20. Lima SL, Dill LM. 1990 Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool*. **68**, 619–640. (doi:10.1139/z90-092)
21. Werner EE, Peacor SD. 2003 A review of trait-mediated indirect interactions in ecological communities. *Ecology* **84**, 1083–1100. (doi:10.1890/0012-9658(2003)084[1083:AROTII]2.0.CO;2)
22. Vonesh JR, Buck J. 2007 Pesticide alters habitat selection in gray treefrogs. *Oecologia* **154**, 219–226. (doi:10.1007/s00442-007-0811-2)
23. Staats EG, Agosta SJ, Vonesh JR. 2016 Data from: Predator diversity reduces habitat colonization by mosquitoes and midges. Dryad Digital Data Repository. (http://dx.doi.org/10.5061/dryad.2f452)

BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Correction

Cite this article: Staats EG, Agosta SJ, Vonesh JR. 2017 Correction to 'Predator diversity reduces habitat colonization by mosquitoes and midges'. *Biol. Lett.* **13**: 20170034. http://dx.doi.org/10.1098/rsbl.2017.0034

Community ecology

Correction to 'Predator diversity reduces habitat colonization by mosquitoes and midges'

Ethan G. Staats, Salvatore J. Agosta and James R. Vonesh

EGS, 0000-0002-5801-6756

Biol. Lett. **13**, 20160580. (Published online 21 December 2016) (doi:10.1098/rsbl.2016.0580)

The penultimate sentence in the Results section contains an error. The sentence '. . . declined 44%, 48% and 51% in the predator-free control . . . ' should instead read '. . . declined 44%, 48% and 51% in the high-richness treatment . . . '. The correct sentence is given here in full:

> Total dipteran oviposition declined 37% in response to dragonflies, 31% to damselflies and 27% to crayfish, relative to predator-free control, and declined 44%, 48% and 51% in the high-richness treatment relative to dragonfly, damselfly and crayfish treatments, respectively (table 1 and figure 1*e*).

This does not affect our results or conclusions. We apologize for any confusion caused.

THE ROYAL SOCIETY PUBLISHING